MORPHOLOGICAL, MOLECULAR AND NUTRITIONAL CHARACTERIZATION OF INDIGENOUS POPULATION OF ATRIPLEX HORTENSIS L. FROM TRANS HIMALAYAN LADAKH REGION OF INDIA

SYNOPSIS

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By

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Introduction

Atriplex hortensis L. Is a member of family of chenopodaceae It ia a annual herb known as mountain spinach, garden orach and sea purslane and locally known as *Phaltora* and *Ustak* in Ladakh region (Rinchen and Narendra 2015). The plant is known to have originated from Europe and Siberia. In traditional medicine, Atriplex is used as health tonic and it helps in nutrition absorption, digestion and enhances the metabolism (Polunin 2009). Beside vitamin and protein the leaves of Atriplex is also rich in calcium, fat, total carbohydrate, fiber, β-carotene, saponin (Sarwa 2001; Duke 1977). Leaves are diuretic, emetic purgative and efficacious when used externally in the treatment of gout. Liniment prepared from the whole plant is used in folk medication such as indurations and tumour (Grieve 1930). It is characterized by a high content of flavonoid, mineral components and amino acids (Steinbach 1996; Hegnauer 1989; Nicol 1994; Siddiqui et al., 1994).

Trans-Himalayan region of Ladakh supports temperate, sub-alpine and alpine vegetation. The vegetation of the cold desert comprises of herbs, shrubs and rarely trees. The vegetatition in the alpine region with exposed moist and boulders habitat, mainly dominated by annual and perennial herbs. The indigenous leafy vegetables (ILV) of Ladakh region include *Amaranthus cruentus, Lepidium latifolium, Rhodiola heterodenta, Fagopyrum tataricum, Fagopyrum esculentum, Atriplex hortensis, Malva vertisilester, Rumex patientia* etc., which are different tribal people of the region (Rinchen et al., 2017). Traditionally, people living in cold desert of Ladakh depend on wild edible plants, including indigenous leafy vegetables (ILF), to meet their dietary requirements. They collect such plants from mountain slopes and around agricultural fields. The indigenous plants do not require intensive care and can grow in less fertile soil. Besides, it can withstand harsh climatic conditions due to its abiotic and biotic stress tolerance potential, which is inherent in their genetic makeup by years of natural selection. However, such plants grow quite sparsely in the nature and their production is very low (Shrawan et al., 2011). Increasing production systems and their inclusion in commonly grown vegetable types in the remote regions would contribute towards nutritional security in the mountainous region.

Among these wild edibles, species found in Ladakh Atriplex *hortensis* is the first green to appear after the prolonged winter (Rinchen and Narendra 2015). Inspite of its potential as leafy vegetable there has been limited or no study carried out on agromorplogical characterization, genetic diversity, phytochemical and nutritional aspects and of this indigenous plant. Therefore it is very important to document the species, its populations in different localities of the region on the basis of agro-morphology, nutritional and genetic diversity in order to establish production, cultivation system and initiate crop improvement programs.

Figure 1.2.1 *Atriplex hortensis* growing in the field (A) Vegetative stage (B) Flowering stage (C) Seed stage.

Literature on related edible plants of Ladakh and their indigenous uses are widely explored. But it was observed that only partial ethno-botanical investigation had been carried out in the region in spite of the huge potential of edible plants as bio-resource. List of some different ethno botanical survey on edible indigenous vegetables conducted in Ladakh is presented in Table 1.1

Table1.1 Ethanobotinical survey carried out in Ladakh, India

Investigator	Plants information	Study site	Observations
Ballabh et al., 2007	44 species of 20 families	Five valleys of Ladakh Zanskar, Drass, Suru, Indus, and Changthang valleys	Raw edibles used by ethnic people of Ladakh
Murugan et al. 2010	27 plant species from 5 genera of 18 families	Nubra valley	Edibles plants used in different Ladakhi dishes
Juliane Dame and Marcus Nusser. 2011	cabbage, cereals, potatoes, turnip, green leafy vegetables and carrots, onions	Five valleys of Ladakh Drass, Zanskar, Indus, Changthang valleys and Suru	The plants are being used in different parts of Ladakh
Alessandro Boesi 2014	12 plants species from 11 genera of 10 families	Sapi (hotspot) region Ladakh	The plants are being used by different tribes of the region as edibles with different recipe

Agro-morphological traits are very common way to estimate relationships between and among genotypes (Bajracharya et al., 2006). Traditional knowledge of existing diversity in indigenous species is useful for germplasm management and conservation. Besides, it is also a very important way to select the parent line having variability within species. Basant and Chaursia 2007 reported that indigenous leafy vegetable *Atriplex hortensis* is being used by local people in Ladakh region. However a comprehensive study on agro-morphological traits of *Atriplex hortensis* L. related to its populations is still not done so far.The present study was undertaken with the objective to identify and evaluate agro morphological traits of *Atriplex hortensis* and its available germplasm in Ladakh region.

Minerals are essential for a wide range of physiological functions in higher animals. Fourteen trace elements including iron have been identified as essential to humans (Zanat et al., 2003). The most widely recognized micronutrient inadequacies are Fe, Zn, and I, yet certain population experience the ill effects of deficiencies of Mg, Ca, and Se. It has been assessed that almost 3.7 billion individuals worldwide are Fe lacking (60%) and that 54% of these 3.7 billion individuals are seriously inadequate (Kachout et al., 2012). There are various reports on studies conducted on mineral composition of different species of *Atriplex* worldwide viz. Khalil et al., 1984 Niekerk et al. 2004, Kachout et al., 2012, Carlson and Hallqvist 1981 Carlson and Clarke 1983. However, these previous studies did not investigate

the mineral compositions of *Atriplex hortensis* and variations in mineral composition in different regional populations. Therefore, the present study was aimed to estimate the mineral content of *Atriplex hortensis* and its populations in the Ladakh region populations. Such selected populations may serve as potential genetic resources for selection of genotypes with higher essential mineral nutrients which could be directly recommended for cultivation or may be included in future breeding programs.

Understanding of genetic diversity assist in management programs viz., conservation and evolutionary history of the species (Nag et al., 2015; Zhai et al., 2016). Molecular markers (DNA) markers are now a day are being routinely used to in assessment of genetic variability of both animal and plant species. Many authors worked on genetic diversity in Ladakh primarily on trees, shrubs and medicinal plants such as, Korekar et al., 2013 *Hippophae rhamnoides,* Prabodh et al., 2014 on *Morus alba.* The assessment of genetic diversity of this indigenous plant species in different population of Ladakh region would also facilitates in devising better conservation strategy and utilization of genetic resources in future breeding programs.

The increasing demand indigenous vegetables could not fulfilled by harvesting these from sparse wild populations that vary in quality and quantity but through cultivation of the selected potential germplasm of the species. Moreover, for the success of commercial cultivations programmes, improvement of selected genotypes with respect to important characters that dictate yield and quality is required. This potential of future improvement is determined by the extent of genetic variability available in the germplasm of a species. Therefore, simultaneous employment of morphological phytochemical, nutritional characteristization and assessment of population genetic diversity would be helpful to recommend potential genotypes / populations with desirable characters for the cultivation as well as to be included in future breeding programs. Thus considering research and development requirements as highlighted above present study was intended to carry out an extensive field survey, agro-morphological and nutritional characterization and to study genetic diversity of *Atriplex hortensis.*

Objectives

1. Screening of selected potential indigenous leafy vegetables of cold desert- Ladakh on basis of yield parameters and consumer acceptance
2. Agro-morphological characterization of indigenous populations of *Atriplex hortensis* L. in Ladakh region.

3. Determination of minerals composition of indigenous populations of *Atriplex hortensis* L. in Ladakh region.
4. Assessment of genetic diversity of *Atriplex hortensis* L. among indigenous populations of Ladakh using RAPD markers.

Objective I

Screening of selected the potential indigenous leafy vegetables of cold desert- Ladakh on basis of yield parameters and consumer acceptance

Materials and Method

Plant material and morphological characters: Seeds of *F. tataricum, R. patientia, F. esculentum, A. cruentus, M. vertisilester, L. latifolium* and *A. hortensis* collected from farmers in Ladakh were sown in a randomized complete block design (RBD) in May 2014 and 2015 on experimental field of DIHAR, Leh, Ladakh, N34º08.516′ and E077º 34.554′ (elevation 3344 m). Agrop-morphological characters were recorded as per descriptor developed by NBPGR, National Bureau of Plant Genetic Resource with slight modification (Singh et al., 2003). The data were recorded by taking three replications of each accession for nine parameters. Qualitative characters were recorded for variation after every 10 days interval at vegetative and flowering stages. Leaf area was recorded using portable laser leaf area meter (CI-201, CID Bio-Science). Petiole length was recorded by cutting portion of the leaf from the base of the leaf blade, and leaf thickness was measured using a digital vernier calliper (MITUTOYO, Japan). Measurement of plant height was done by using a measuring scale. Shoot diameter was recorded by taking three readings from the lower, middle and top of the shoot. Leaf chlorophyll contents were measured with a chlorophyll content meter (CCM-200plus, Opti-Sciences, Inc., USA).

Cultivation practices: During the time of field preparation Farm yard manure (25 $t.ha^{-1}$) was used. Flat bed 2 m wide and 2 m long were prepared. Seeds were mixed with sand in 3:1 ratio and sown in rows 20 cm apart. Plant to plant distance was maintained at 12 cm by thinning. Irrigation was done by flooding immediately after sowing followed by seven days interval at later stages. Weeding was done manually. Additional FYM (1.5 $kg.m^2$) was applied at the time of hoeing after first picking of leaf. Leaf harvest was made by cutting the plant few centimetres above the ground except in case of *M. vertisilester* where it was harvested by topping leaves at middle of petiole.

Organoleptic test: Forty-five trained subjects which were healthy and non-smokers in the age bracket of 20 to 55 years, comprising of 20 female and 25 male were selected to participate in the organoleptic test trials. The subjects were asked to refrain from eating or drinking for a minimum of one hour prior to their individual testing sessions. On the day of testing, freshly harvested leaves were washed and trimmed before preparation. It was cooked and a pinch of salt was added. A stir bar was used to mix the salt until it was completely dissolved, and then promptly store in a containers at room temperature. Each subject was asked to score the samples on 9-points hedonic scale on paper ballot (Peryam and Pilgrim 1957). In between each sample, panellists were asked to rinse their mouth at least twice with water during a one minute break. A five minute break was given between testing blocks to prevent fatiguing.

Data analysis: The data was evaluated for ANOVA, one way analysis of variance. Correlation and HSD, two-sided Tukeys Honestly Significant Difference at $p \leq 0.05$ was carried out using SPSS version 17.0.

Results

Crop yield potential: The yield potential of ILVs are presented in Table 1. Significant difference in yield potential was observed which ranged from 0.45 to 1.86 kg.m^2. *A. hortensis* showed the highest yield (1.86 kg.m^2) followed *F. tataricum* (1.75 kg.m^2) and *R. patientia* (0.86 kg.m^2). The crops attain harvesting stage between 25 to 85 days after sowing. Large variation in numbers of days to harvesting ensured availability of the ILV for a longer duration in the harsh environmental conditions.

Table 1: List of indigenous leafy vegetables of trans-Himalayan Ladakh and yield potential

Botanical name	Vernacular name	Common name	Days of harvesting	Yield (kg.m^2)
Amaranthus cruentus	Khi snama	Amaranth	60-70	0.65±0.38^{b}
Atriplex hortensis	Phaltora/Phaltor	Mountain orach	25-30	1.80±0.06^{d}
Fagopyrum esculentum	Tayat/bro	Buckwheat	45-50	0.75±0.03^{bc}
Fagopyrum tataricum	Tayat/Kho bro	Buckwheat	50-55	1.75±0.04^{d}
Malva vertisilester	Sochilik	Chinese mallow	75-85	0.64±0.35^{ab}

Rumex patientia	Shoma	Garden patience	70-80	0.86±0.33^{c}
Lepidium latifolium	Shang sho	Pepper weed	55-68	0.45±0.43^{a}

Significantly different at $p < 0.05$ for each column is indicated by lowercase letters

Morphological characters: Significant variation in plant morphological characters were observed between the seven ILVs studied (Table 2). Plant height ranged from 9.0 to 19.5 cm depending on the crop. The mean number of leaf ranged from 6.3 to 14.7 per plant. Leaf characteristics and petiole length showed significant differences among the seven ILVs studied. The chlorophyll contents ranged from 28.9 to 49.4 mg.g^{-1}.

Table 2: Morphological characters of indigenous vegetables of trans-Himalayan Ladakh

Characters	*F. esculentum*	*A. hortensis*	*F. tataricum*	*M. vertisilester*	*R. patientia*	*L. latifolium*	*A. cruentus*
Plant height (cm)	19.50±2.02^{c}	12.40±0.55^{ab}	16.87±0.03^{bc}	13.40±0.95^{ab}	9.00±0.58^{a}	13.00±0.58^{ab}	11.33±0.88^{a}
No. of leaf/plant	7.33±0.33^{ab}	14.00±2.00^{bc}	8.00±2.31^{abc}	6.33±1.20^{a}	14.67±1.45^{c}	9.76±0.88^{abc}	14.67±1.45^{c}
Leaf length (cm)	2.86±0.49^{a}	4.18±0.87^{ab}	5.61±0.32^{abc}	4.19±0.17^{ab}	6.49±0.32^{bc}	7.72±1.26^{c}	3.89±0.90^{ab}
Leaf width (cm)	3.33±0.44^{ab}	3.92±0.97^{abc}	5.87±0.23^{c}	4.66±0.12^{bc}	2.43±0.19^{a}	2.98±0.17^{ab}	2.43±0.19^{a}
Leaf thickness (mm)	0.48±0.11^{a}	0.50±0.13^{a}	0.48±0.02^{a}	0.32±0.01^{a}	0.24±0.03^{a}	0.49±0.14^{a}	0.30±0.03^{a}
Petiole length (cm)	3.97±0.26^{bc}	1.83±.75^{ab}	7.90±1.30^{c}	5.77±0.93^{bc}	3.33±1.42^{ab}	0.10±0.00^{a}	1.30±0.35^{a}
Leaf area (cm^2)	6.67±1.47^{a}	10.27±4.41^{a}	16.58±7.06^{a}	12.64±1.06^{a}	11.43±1.27^{a}	14.98±3.16^{a}	8.77±0.41^{a}
Chlorophyll (mg.g^{-1})	28.90±0.89^{a}	55.33±2.02^{d}	38.67±2.70^{ab}	42.67±3.28^{bc}	49.37±2.18^{cd}	45.00±1.15^{bcd}	49.37±2.18^{cd}

Representing the values as mean ± SD

Significantly different at $p < 0.05$ for each column is indicated by lowercase letters

Correlation among variables: Table 3 present correlations among variables. Significant correlation was observed between plant height with leaf width (r = -0.435), yield (r = -0.540) and leaf length (r = 0.438). However, none of the values were highly significant. Number of leaf per plant is considerably correlated with thickness of leaf (r = 0.537) and chlorophyll contents (r =-0.789). Length of Leaf is correlate positively with chlorophyll contents (r = 0.592), and correlate negatively with width of leaf (r = -0.509). Leaf thickness is correlate positively with length of petiole (r = 0.671), leaf area (r = 0.515) and yield (r = 0.549).

Table 2.3.3.1 Pearson's correlation coefficients of morphological traits, chlorophyll contents and yield of indigenous leafy vegetables of trans-Himalayan Ladakh.

Variables	Plant height	Leaf/ plant	Length of leaf	Width of leaf	Thickness of leaf	Length of petiole	Area of leaf	Chlorophyll content	Yield
Plant height	1	0.356	0.438*	-0.435*	-0.338	-0.410	0.129	0.429	-0.540*
Leaves/plant		1	-0.335	0.325	0.537*	0.418	-0.081	-0.789**	0.141
Length of leaf			1	-0.509*	-0.095	-0.412	-0.069	0.592**	0.112
Width of leaf				1	-0.091	-0.170	0.616**	0.297	-0.099
Thickness of leaf					1	0.671**	0.515*	-0.172	0.549**
Length of petiole						1	-0.097	-0.240	0.235
Area of Leaf							1	-0.372	0.367
Chlorophyll content								1	0.139
Yield									1

*****Significant = $p \leq 0.05$, ****** Significant =$p \leq 0.01$

Organoleptic test: Consumer preference for ILVs based on organoleptic test and its comparison with spinach is presented in Table 4. *A. hortensis* was preferred over all other leafy vegetables in terms of taste, flavour, palatability and overall acceptability. *A. crunteus* was at par with the spinach in terms of taste, flavour, palatability and overall acceptability. Other ILVs ranked lower in terms of acceptability as compared with spinach. Therefore, *A. hortensis* and *A. crunteus* need to be studied in details and promoted among the people living in the mountain terrain.

Table 4: Organoleptic test for preference of indigenous leafy vegetables of trans-Himalayan Ladakh in comparison to spinach

Vegetable	Taste	Flavour	Palatability	Overall

				acceptability
Spinach	7.50±0.43^{bc}	7.33±0.49^{bc}	7.00±0.36^{bc}	7.33±0.49^{bc}
Amaranthus crunteus	7.33±0.49^{bc}	7.17±0.48^{bc}	7.17±0.48^{bc}	7.00±0.45^{bc}
Fagopyrum tataricum	7.33±0.75^{bc}	6.83±0.40^{bc}	6.50±0.50^{abc}	6.67±0.61^{bc}
Malva vertiselester	6.17±0.21^{b}	5.67±0.61^{ab}	5.67±0.92^{ab}	5.17±1.11^{ab}
Atriplex hortensis	8.67±0.45^{c}	8.33±0.21^{c}	8.33±0.33^{c}	8.33±0.33^{c}
Fagopyrum esculentum	6.00±0.60^{b}	5.83±0.48^{ab}	5.83±0.60^{abc}	5.83±0.60^{abc}
Rumex patientia	5.83±0.33^{b}	5.33±0.76^{ab}	5.50±0.67^{ab}	5.50±0.67^{abc}
Lepidium latifolium	3.33±0.27^{a}	4.00±0.26^{a}	4.33±0.33^{a}	3.12 ±0.60^{a}

Representing the values as mean ± SD

Significantly different at $p < 0.05$ for each column is indicated by lowercase letters

It was suggested in the study that *A. hortensis* and *A. crunteus* were most preferred vegetables by consumer and have high palatability. *A. hortensis* is early maturing crop and its yield potential is significantly higher. Therefore, a detailed study on *A. hortensis* is required along with efforts for its large scale cultivation.

Objective II

Agro-morphological characterization of indigenous populations of *Atriplex hortensis* L. in Ladakh region.

Material and methods

Experimental site: The experiments were conducted in experimental field of Defence Institute of High Altitude Research (DIHAR) Leh, Ladakh, Jammu and Kashmir, India. Representative germplasm of *A. hortensis* (N=132) from random wild and cultivated field, representing three natural populations, were collected from 15 different collection sites spread across Indus, Suru and Nubra valleys in the Trans-Himalaya region in Ladakh during 2013-2014 (Table 2.1). Three representatives of plants were selected randomly for each accession for recording observations on twenty one traits. The valleys were considered as separate populations. The altitude of collection sites ranged from 2500-4101m amsl. Altitude and location of study sites were established using GPS (GARMIN 72, Olathe, Kansas USA). Precipitation of the study region is less than 200 mm of which more than 70% is in the form of snow (Korekar et al., 2013). A herbarium of *Atriplex* representative samples collected from three valleys was prepared and the voucher specimens were submitted to Botanical Survey of

India (BSI), Dehradun, India, to ascertain the Atriplex species status. Collected accessions of *A. hortensis* were grown in summer cropping season. with three replications established in randomized blocks, a total of three hundred ninety six experimental beds were prepared of size $2m^2$ (1m x 2 m) with three rows with spacing of 30 cm between rows and 15 cm between plants. The crop received a basal dose of locally available compost and irrigations (Figure 2.1). The crop was protected from weeds by diweeding.

Table 2.1 Geographical location and sampling sites of *Atriplex hortensis* in the Trans-Himalaya region of Ladakh.

Valleys	Sub -pop	Accession no. (s)	Alt. (m)	Lat (N)	Long (E)
Nubra	Khardong	6	4109	34° 33.822	077° 39.487
	Tangyar	10	3915	34° 15.168	077°52.259
	Khungru	9	3623	34° 17.003	077° 26.556
	Agham	11	3335	34° 19.706	077° 49.877
	Murghi	5	3201	34° 45.516	077° 32.643
	Panamik	5	3184	34° 46.963	077° 32.304
	Hunder	20	3169	34° 35.213	077° 27.688
	Udmaru	5	3129	34° 37.627	077° 26.180
	Diskit	5	3117	34° 33.210	077° 32.686
Indus	Gonpa	10	3666	34° 11.026	077° 35.560
	Shenam	11	3477	34° 09.550	077° 34.766
Suru	Chiktan	11	3252	34°28.166	076°30.432
	Achakmal	6	2888	34°33.220	076°09.449
	Pakskum	11	2881	34°33.120	076°09.443
	Soad	7	2860	34°33.974	076° 10.305

Note: Sub pop-Sub populations; No.-Number of samples; Alt-Altitude (m asl); Lat-Latitude; Long-Longitude.

Figure 2.1 *Atriplex hortensis* plants collected from different collection sites grown in experimental field.

Quantitative and qualitative characters: Morphological data were recorded taking three replications of each accession for twenty one characters belonging to six qualitative traits and fifteen quantitative traits (Table 2.2**)**. To study the morphological characters, we used a descriptors developed by National Bureau of Plant Genetic Resource (NBPGR) (Singh et al. 2003). All qualitative and quantitative traits were regularly recorded for variation in characters after an interval of 10 days at peak of the vegetative and flowering period. Characterization of plants were carried out on the basis of recorded data of vegetative and inflorescence characters that recorded for both qualitative and quantitative traits at different stages of plant growth, vegetative data collected in 35-40 days when the plant is ready to consume, flowering data collected at 55-65 and at fruiting stage of 85-95 days old plant. Data recorded from three replications and analysed statistically. Leaf length, leaf width, and leaf area were recorded using portable laser leaf area meter (CI-201, CID Bio-Science), petiole length by cutting portion of the leaf from the base of the leaf blade, leaf thickness by digital vernier calliper (MITUTOYO, Japan) measured at lower, middle and top of the leaf blade and average was recorded, leaf aestivation was recorded from vegetative to reproductive stage, number of leaf per plant and weight were recorded at vegetative stage when the plant was ready to harvest for consumption, moisture content was recorded by wet weight of 100 g minus dry weight using digital electronic weighing balance to an accuracy of 0.01 g, plant

height was measured from the base of the plant to tip of the leaf using measuring scale, shoot diameter was recorded by taking three readings from the lower middle and top of the shoot using digital vernier calliper (MITUTOYO, Japan), leaf colour was recorded by colour chart, seed size was measured by using digital vernier calliper (MITUTOYO, Japan), total soluble solid (TSS) concentration of freshly- chopped extracted juice of representative leaf samples was determined with a digital hand refractometer (Atago, ATC-1E, Kyoto, Japan) (Ozgen and Sekerci 2011). The leaf chlorophyll content of the species was obtained by collecting three leaf sample of the same size with three replications using chlorophyll content meter (Opti-science, CCM-200 plus).

Tablec2.2 Qualitative and quantitative characters of *Atriplex. hortensis* used for data recording in the study

S. No.	Qualitative characters	S. No.	Quantitative characters
1	Total soluble solids (Brix %)	1	Plant height (cm)
2	Chlorophyll content (CCI unit 0.71mm^2)	2	Shoot diameter (mm)
3	Moisture content (%)	3	Leaf lamina length (cm)
4	Leaf colour (colour chart)	4	Leaf lamina width (cm)
5	Dry weight (g)	5	Leaf lamina area (cm^2)
6	1000 seed weight (g)	6	Leaf thickness (mm)
		7	Petiole length (cm)
		8	Primary branches
		9	Number of Inflorescence
		10	Inflorescence length (cm)
		11	Seed size (mm)
		12	Seed yield (g)
		13	Number of leaf per plant
		14	Leaf yield per plant
		15	Leaf aestivation

Statistical analysis: Univariate and multivariate statistical procedures were used to analyse the data taken during the study using SPSS 17.0 software. Analysis of variance (ANOVA) was performed and the mean of the results were compared by Duncan's multiple range tests at 5% significance level. To determine the degree of associations among the characters, Pearson's correlation analysis was performed. Principal component analysis (PCA) and

multidimensional scaling (MDS) was used to ordinate population means considering variance and covariance among characters within and among populations (Kim 1975). Average Euclidean distance was calculated for each population and the resulting distance matrix was used to construct a phenetic dendogram using average linkage method (Mohammadi and Prasanna 2003).

Results

Variation in morphological characters: Considerable variations were observed on various morphological characters of the species collected from different geographical region. Leaves colours - dark green, intermediate and light green were observed at higher, middle and lower altitude accessions respectively. The leaf margins (dentate, smooth) and aestivation (lower opposite decussate and upper opposite) were observed scattered among all the populations. The morphological characters (Table 2.4) recorded in the study showed that the mean plant height ranged from P≥0.001 (15.36 to 27.35 cm), while mean leaf length varied from P≥ 0.002 (6.39 to 11.46 cm), mean leaf width was p≥0.013 (4.82 to 6.46 cm), mean leaf area was P≥ 0.001 (20.22 to 46.28cm^2), mean number of leaf was P≥ 0.013 (18.27 to 37.93), mean chlorophyll content was P≥0.005 (43.19 to 70.60mm^2), mean dry weight was P≥ 0.026 (11.09 to 12.64g), mean shoot diameter was P≥ 0.026 (4.07 to 5.38mm), mean petiole length was P≥ 0.003 (2.47 to 3.76cm), mean moisture content was P≥ 0.02 (87.36 to 88.91%), mean inflorescence per plant was P≥ 0.017 (20.17 to 29.47), mean seed yield per plant was P≥0.26 (32.25 to 67.39g) and mean 1000 seed weight was ranged between P≥ 0.001 (1.91 to 2.20 g) in the study area. The highest values of mean chlorophyll content, number of leaf per plant, mean leaf area, mean leaf length, mean petiole length were found in Panamik, mean plant height, mean primary branches per plant found in Udhmaru and mean seed yield per plant, and 1000 seed weight were found in Murghi collection site of Nubra valley area. On the other hand highest mean dry weight and mean shoot diameter were recorded in Shenam and Gonpa sites of Indus valley. These five populations were superior and significant different as compare to other populations at 5% level Figure 2.2 and 5 show the character values plotted against the first two principal component variants from PCA and sample population with respect to their Euclidean distance from MDS. Morphological characters are based on phenotype expression of the population and environmental factors (Heywood 2002)**.** Traits are positioned quite close to each other in the axis with respect to their population (four groups) (Figure 2.3). The closely related samples belong to group II which including Akchamal, Diskit, Tangyar, Agyam, Khungru, while group I including Panamik, Pakskum,

Shenam, Chiktan, group III including Soad, Gonpa, Udmaru and group IV including Khardong, Hunder and Murghi populations. The most significant morphological characters were distinguish from these four groups are reflected in their loadings on the first two principal components. The first two principal components include more than 60 % variation among the populations. The highest loadings characters are, number of leaf per plant, leaf area, leaf width, plant height, petiole length, leaf yield per plant, shoot diameter and number of inflorescence for principal component 1 where as seed size and number of primary branches for principal component 2 (Table 2.3).

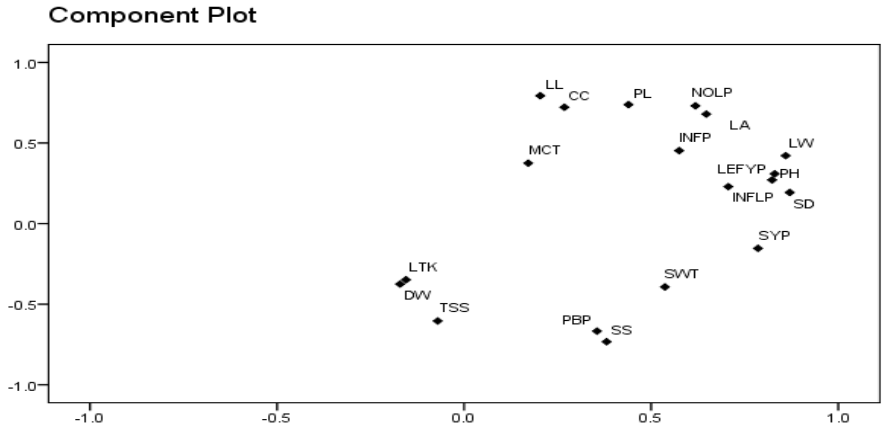


Figure 2.2 Principal component analysis of morphological data of *Atriplex hortensis* (NOLP: Number of leaf per plant, LA: Leaf area, LW: Leaf width, PH: Plant height, PL: Petiole length, LEFYP: Leaf yield per plant, SD: Shoot diameter, INFP: Number of Inflorescence, INFLP: Inflorescence length, CC: Chlorophyll content, LL: Leaf length, TSS: Total soluble solids, DW: Dry weight, MC: Moisture content, LTK: Leaf thickness, SS: Seed size, PBP: Number of primary branches per plant, SWT: 1000 seed weight, SYP: Seed yield per plant.

Table 2.3 Morphological characters of *Atriplex hortensis* and their principal component weightage recorded during the study

Characters	**Characters acronym**	**PC1 (42.50 %)**	**PC2 (16%=59.59)**
Number of leaf per plant	NOLP	0.944	-0.159
Leaf area	LA	0.933	-0.101
Leaf width	LW	0.929	0.233
Plant height	PH	0.833	0.300
Petiole length	PL	0.812	-0.280
Leaf yield per plant	LEFYP	0.803	0.325
Shoot diameter	SD	0.789	0.414
Number of Inflorescence	INFP	0.731	0.025
Inflorescence length	INFLP	0.687	0.281
Chlorophyll content	CC	0.670	-0.378
Leaf length	LL	0.667	-0.475
Total soluble solids	TSS	-0.444	0.416
Dry weight	DW	-0.373	0.176
Moisture content	MCT	0.373	-0.176
Leaf thickness	LTK	-0.344	0.166
Seed size	SS	-0.182	0.805
Number of Primary branches per plant	PBP	-0.159	0.739
1000 seed weight	SWT	0.156	0.647
Seed yield per plant	SYP	0.501	0.624

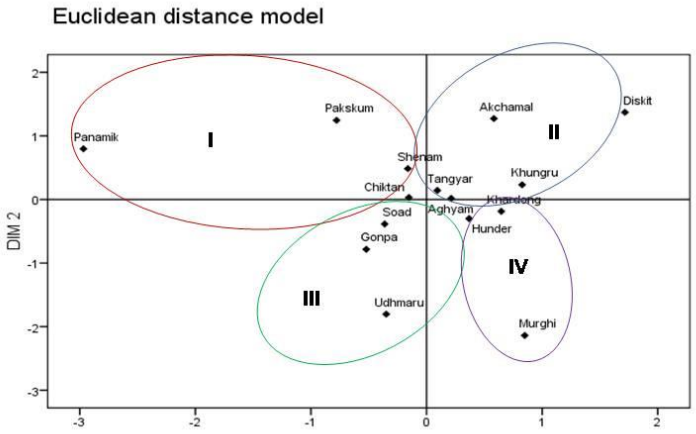


Figure 2.3 Multidimensional scaling of morphological data of *Atriplex hortensis* with their respective populations.

Dendrogram was drawn to show the phenetic relationships among different populations based on Euclidean distances from morphological data matrix (Figure 2.4). Dendrogram grouped 396 phenotype into main clusters A and B. Cluster A represent the phenotype of Aghyam, Hunder, Tangyar, Chiktan, Pakskum, Gonpa, Khardong, Shenam, Murghi, Soad, Udhamaru, and Panamikwhile, while the cluster B represent the phenotype of Khungru, Diskit and Akchamal. The results of PCA and MDS analysis were comparable to the cluster analysis.

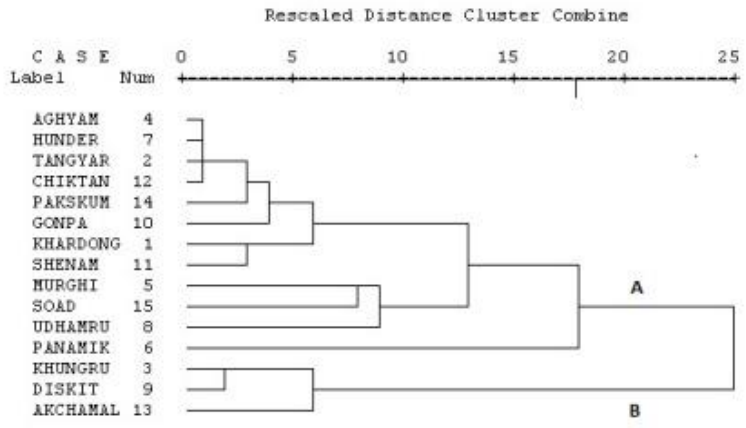


Figure 2.4 Dendogram showing the phenetic relationship among 15 populations based on Euclidean distance from morphological data matrix. Dendogram using Average Linkage (Within Group)

Table 2.4 Duncan's test for mean comparisons of morphological characters among of *Atriplex hortensis*

Population	Mean total soluble solids (SE) brix %	Mean chlorophyll content (SE) SPAD	Mean number of leaf per plant (SE)	Mean Plant height (SE) cm	Mean leaf area (SE) cm2	Mean leaf length (SE) cm	Mean leaf width (SE) cm
Khardong	9.77b (0.51)	53.27abc (5.13)	23.67abc (2.49)	24.74d (1.73)	25.17ab (2.49)	7.80abc (0.53)	5.25ab (1.37)
Tangyar	8.87abc 0.40)	53.45abc (3.45)	27.17abc (1.93)	21.51bcd 1.50)	29.59abc (2.09)	8.90bc (0.45)	5.56abc(1.21)
Gonpa	9.22ab (0.29)	47.25bcd (3.84)	24.00bcd (3.19)	18.48d (1.40)	23.83bc (2.15)	6.78abc (0.48)	4.82abc(1.38)
Khungru	8.56abc 0.31)	51.03ab (3.31)	26.64abc (2.25)	23.15abc 1.52)	29.89ab (2.13)	8.63ab (0.43)	5.60a (1.38)
Aghyam	9.04ab 0.67)	52.25abc (3.36)	27.48abc (3.72)	23.37cd (1.82)	31.42abc (3.59)	8.51abc (0.72)	5.89abc (1.60)
chiktan	8.48abc 0.55)	70.60bcd (4.67)	37.93bc (5.43)	25.97bcd(2.25)	46.28bc (8.48)	11.46bc (1.32)	6.43abc (2.14)
Shenam	8.69abc 0.20)	52.67bcd (1.95)	24.91ab (1.48)	23.74d (1.20)	28.32abc (1.75)	8.56 abc (0.62)	5.43abc (1.42)
Murghi	8.22abc 0.30)	52.87abc (5.38)	32.87abc (4.07)	27.35cd (2.43)	33.09bc (2.79)	8.60 abc (0.42)	6.30abc(1.33)
Panamik	9.87ab (0.91)	43.19d (4.47)	18.27d (1.75)	15.36d (1.97)	20.22abc (3.20)	6.39 d (0.60)	4.97c (2.35)
Hunder	8.43ab (0.33)	58.09abc (3.65)	30.03abc (2.62)	25.30cd (1.95)	31.77abc (2.47)	8.69 abc (0.52)	5.89abc (1.60)
Udmaru	8.85a (0.33)	59.51abc (3.71)	23.18cd (1.90)	25.46d (1.24)	28.06dc (2.32)	8.17 abc (0.50)	5.45bc (1.40)
Diskit	9.11bc (0.30)	57.50a (4.13)	28.77a (2.76)	21.87a (1.48)	30.64a (3.35)	8.74a (0.64)	5.61a (1.97)
Ackchamal	8.75ab (0.37)	53.78abc (5.96)	22.50ab (2.89)	16.44ab (1.54)	23.41ab (2.32)	7.53abc (0.52)	4.85a (1.23)
Pakskum	9.25abc 0.50)	65.16cd (4.50)	26.34abc (3.08)	23.60cd (1.44)	28.81abc (3.10)	8.18abc (0.59)	5.45abc(1.88)
Soad	10.25c (0.51)	55.24abc (4.62)	28.71bc (2.13)	26.68d (2.17)	36.22c (4.14)	9.61cd (0.71)	6.46c (1.85)
Population	Mean leaf thickness (SE) mm	Mean dry weight (SE) g	Mean shoot diameter (SE) mm	Mean petiole length (SE) cm	Mean leaf yield per plant (SE) g	Mean primary branches per plant (SE)	Mean moisture content (SE) %
khardong	0.60a (0.02)	12.46bc (0.56)	4.66abc (0.25)	2.59a (0.16)	16.33abc (2.24)	26.72bc (1.67)	87.54ab (0.56)
Tangyar	0.64a (0.02)	12.04abc (0.20)	4.87abc (0.21)	2.80ab (0.12)	18.91abc (2.11)	26.23abc (1.38)	87.96abc (0.20)
Gonpa	0.64a (0.08)	12.27ab (0.36)	4.07c (0.23)	2.58ab (0.12)	13.99c (2.02)	23.44abc (1.55)	87.73bc (0.36)
Khungru	0.62a (0.03)	11.74bc (0.16)	4.67a (0.18)	2.58a (0.11)	18.82ab (1.90)	24.64ab (1.49)	88.26ab (0.16)
Aghyam	0.71a (0.09)	12.31abc (0.28)	4.83abc (0.39)	2.62a (0.20)	20.58abc (3.33)	29.47abc (1.13)	87.69abc (0.28)
chiktan	0.61a (0.06)	11.95abc (0.47)	4.85abc (0.31)	3.76ab (0.50)	19.60abc (2.89)	20.40abc (2.01)	88.05abc (0.47)
Shenam	0.64a (0.02)	11.94c (0.18)	4.80abc (0.14)	2.78ab (0.09)	18.54abc (1.68)	22.98ab (0.97)	88.06a (0.18)

Murgi	0.60a (0.03)	11.96bc (0.34)	5.08abc (0.33)	3.04ab (0.14)	23.44abc (4.03)	23.00c (2.99)	88.04ab (0.34)
Panamik	0.67a (0.05)	12.63abc (0.24)	4.27abc (0.33)	2.47c (0.24)	11.61abc (1.98)	23.93a (1.62)	87.37abc (0.24)
Hunder	0.66a (0.04)	11.50abc (0.23)	5.38abc (0.27)	2.81ab (0.16)	26.84abc (5.73)	24.53ab (1.17)	88.50abc (0.23)
Udmaru	0.60a (0.03)	12.64abc (0.24)	4.67bc (0.18)	2.98ab (0.17)	17.54bc (1.84)	20.76ab (1.42)	87.36abc (0.24)
Diskit	0.59a (0.01)	12.01c (0.36)	4.83ab (0.25)	2.66a (0.15)	22.25a (3.75)	24.51abc (2.18)	87.99a (0.36)
Ackchamal	0.67a (0.03)	11.08a (0.38)	4.22ab (0.33)	2.71ab (0.21)	15.12ab (2.51)	23.22ab (1.50)	88.92c (0.38)
Pakskum	0.64a (0.02)	12.03abc (0.20)	4.67abc (0.28)	2.76ab (0.15)	19.12abc (2.83)	21.58ab (1.30)	87.97abc (0.20)
Soad	0.62a (0.02)	11.43ab (0.28)	5.28c (0.29)	3.22b (0.27)	22.76bc (2.47)	25.81abc (2.83)	88.58bc (0.28)

Population	Inflorescence length per plant (SE) cm	Number of inflorescence per plant (SE)	Seed yield per plant (SE) g	Seed size (SE) mm	Seed weight (SE) g
khardong	16.33abc (2.24)	20.17a (2.07)	48.60ab (7.24)	2.05abcd (.06)	2.91ab (0.13)
Tangyar	18.91abc (2.11)	22.33abc (1.39)	41.99a (4.12)	1.99ab (.04)	2.56ab (0.10)
Gonpa	13.99abc (2.02)	23.15abc (1.16)	41.98ab (6.04)	2.03abcd (.05)	2.89ab (0.38)
Khungru	18.82abc (1.90)	25.27abc (1.15)	42.02a (4.63)	1.99abcd (.05)	2.55ab (0.08)
Aghyam	20.58bc (3.33)	25.40abcd (1.86)	67.39a (12.22)	2.20ab (.06)	3.12ab (0.16)
chiktan	19.60abc (2.89)	27.07abc (2.07)	45.36a (6.34)	1.91bcd (.07)	2.64ab (0.14)
Shenam	18.54abc (1.68)	24.10abc (1.07)	47.56a (3.96)	2.09cd (.03)	2.80b (0.07)
Murgi	23.44bc (4.03)	29.47abcd (1.13)	53.88b (9.92)	1.97d (.07)	2.56ab (0.16)
Panamik	11.61bc (1.98)	20.40cd (2.01)	32.25a (6.93)	2.07a (.06)	2.66ab (0.12)
Hunder	26.84c (5.73)	23.27abc (1.59)	54.09ab (7.53)	2.03bcd (.04)	2.68ab (0.09)
Udmaru	17.54d (1.84)	23.18d (1.08)	44.11ab (5.45)	2.19ab (.04)	3.40ab (0.11)
Diskit	22.25a (3.75)	23.15ab (1.58)	45.07a (7.42)	2.10abcd (.05)	2.59ab (0.10)
Ackchamal	15.12ab (2.51)	24.33abcd (1.63)	36.06a (7.73)	2.02abc (.07)	2.45a (0.14)
Pakskum	19.12abc (2.83)	20.33ab (1.35)	38.44a (3.75)	1.98ab (.03)	2.73ab (0.18)
Soad	22.76c (2.47)	25.86bcd (2.65)	45.45a (5.56)	2.20d (.07)	4.15c (0.94)

Objective III

Determination of minerals composition of indigenous populations of *Atriplex hortensis* L. in Ladakh region.

Materials and methods

Study Area: The study was conducted at vegetable research unit (altitude 3500 m msl, latitude 34°8'16.119" N, longitude 77°34'19.2216"E) of Defence Institute of High Altitude Research (DIHAR), in Leh- Ladakh, India. The soil texture of the experimental site was silty loam with pH 7.5±0.3 (mean±SD). Organic matter and organic carbon content were 4.4±0.5% and 1.3±0.2%, respectively.

Experiment design and sampling: To determine the diversity of mineral accumulation, 14 populations of 5 accessions each representing population of *A. hortensis* L. from three valleys (Nubra, Indus and Suru) were collected (Figure 3.1). The samples were carefully collected at a distance of about 2 to 5 km of each accessions and about 25 to 250 km from each population. The seeds were sown in DIHAR farms during May 2016 in randomized block design for the production of vegetative part in open conditions. The plot size was $2m^2$ and row-to-row and plant-to-plant spacing was 15 cm and 10 cm respectively. All recommended agronomic practices were followed. The harvest was carried out approximately after 30 to 45 days when the plants were young and tender and ready to consume. Random samples from each set of three plants leaves from each accession and each replication were combined. Each sample was weighed and immediately snap-frozen in liquid nitrogen, and stored at -80ºC until it was lyophilized. Dry weight measurements were made on lyophilized samples.

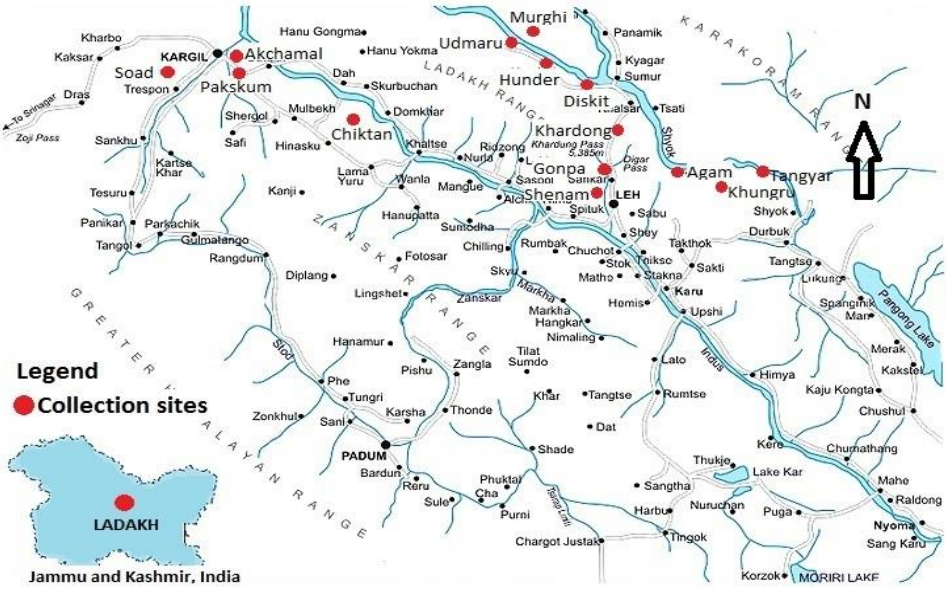


Figure 3.1 Map of Ladakh Region showing the collection sites (marked in red) of *A. hortensis* L. accessions for the present study. (Not to be scaled)

Chemical and reagent: All chemicals and reagents used were of analytical grade. Deionized water (Millipore Water) (RIOs^{TM} type I simplicity, 185 USA) with a resistivity of 18.2MΩ cm and Borosil glassware were used for the preparation of solutions and sample analysis. For mineral analysis, Multielement calibration standard solution IV (Merck, Darmstadt, Germany) was used. All the glassware and were sterilized before used in experiments.

Sample treatment and sample analysis: Mineralization of all the samples was made by hot block digester of Questron Technologies Corporation, Canada. In the test tube 0.5mL of samples were taken and 10mL of Nitric acid (70% HNO_3, Merck, Darmstadt, Germany) was added to the test tube. Tube were left for 30 minutes at room temperature under a fume hood and then heated for 25 min at 70°C temperature. After cooling, 2mL $HCLO_4$ (70%, Merck, Darmstadt, Germany) was added and heated for 25 min at 135°C temperature. After that, 2mL of $HCLO_4$ and 2mL of HNO_3 was added and heated for 25 min at 135°C temperature. Then, 2mL of HNO_3 + 5mL of HCL (37%, Merck, Darmstadt, Germany) were added and the solution was transferred to 50mL falcon tube after cooling. Final volume was adjusted up to 50mL with distilled water (Charan 2013). Thirteen minerals elements were selected for analysis by ICP-OES. Analysis of minerals by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Model no. Optima 7000DV PerkinElmer, USA). Multielement calibration standards were prepared by appropriate dilution of ICP multi-element standard solutions Merck, Darmstadt, Germany. The plasma conditions of ICP-OES method were standardized according to Giri et al., 2017 with some modification.

Statistical analysis: Data have been analysed using analysis of variance (ANOVA) followed by Tukey's test and considered significant if $p \leq 0.05$. Genetic diversity among the populations was studied using generalized distance (D2). Principal component analysis (PCA) was used as correlation matrix to determine the variables containing maximum possible variance with the number of principal components. The similarity coefficient was determined by General Similarity Coefficient that reflects the differences existing among the accessions. All statistical analyses were performed using SPSS version 21.0 (Statistical Program for Social Science, SPSS Corporation, Chicago, Illinois, USA) and XLSTAT, 2017 (Statistical software and data analysis add- on for Excel).

Results

The concentration of 13 important mineral elements namely, Ca, Mg, Zn, Fe, Na, Al, K, P, S, Si, B, Cu and Se in the leaves of the 70 accessions representing 14 different populations of *A. hortensis* L. of Ladakh region have been shown in Table 5.1. Varying levels

of concentration of mineral elements was found in different populations. S, Al, K and Ca were found in high concentration than the other mineral elements (Table 5.1). A highest concentration of S (81.43±0.025 mg/g) was recorded in population collected from Gonpa area whereas the lowest was found in the population collected from Udmaru area (53.09±0.020 mg/g). On the other hand lowest concentration was recorded for Se in the present study. It ranged from 0.004±0.001 -0.089±0.003 mg/g in the study populations. Furthermore, Al was also found in considerably high concentration and ranged between 5.26±0.003 - 12.95±0.006 mg/g in the present study. Among the other elements, K and Ca were present in considerable concentration and their highest concentration of 1.83±0.002 mg/g and 1.70±0.002 mg/g respectively was recorded in the populations collected from Pakskum.

Overall there was variation in the concentrations of mineral elements studied in different populations in the present study and found statistically significant (Table 5.1). The concentrations of some important mineral elements among different populations varied by 1.5 folds for S, 2.8 folds for Al, 1.5 folds for K and 2.4 for Ca respectively. Furthermore, taking into consideration the coefficient of variation, the observed variation for micronutrient was 98% for Fe, 94% for B, 30% for Zn, 10 % for Cu, 54% for Se, whereas for macronutrients was found to be 24% for Mg, 11% for K, 13% for Na and 27% for Ca respectively

A total correlation matrix analysis (Table 5.2) revealed two strong sets of correlations, one was found associating Ca, Mg, Zn, Na, K, S, B and Cu ($p \leq 0.01$), while the other to Si ($p \leq 0.05$). PCA showed that Mg, Na, Zn, Si and Ca show highly correlated to first component (27% of the variability), K, Cu and P were correlated to the second component (17% of the variability), and Al, S to the third component (15% of the variability) (Figure 5.2)

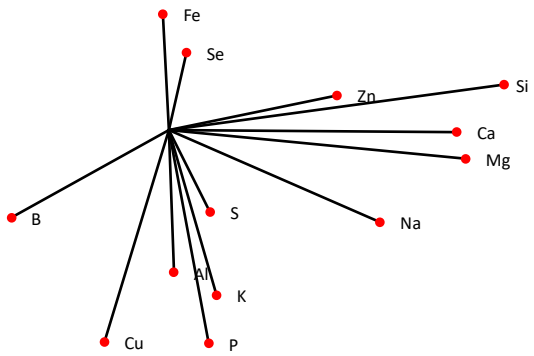


Figure 4.3.1 Principal Component Analysis (PCA) of the mineral elements analysed in *A. hortensis* L. accessions in the study.

Ladakh is a cold desert region which remains mostly inaccessible during the winter season, hence civil as well military population inhabiting the region remains deprived of fresh vegetables during the season. The region does not support the cultivation of vegetables in more than 6 months long chilling winters except the few, among which *A. hortensis* L. is considered important. However the nutritional importance of this vegetable has not been studied properly to make this more popular atleast during the un-favourable season. Present results have demonstrated that *A. hortensis* L. is found in majority of the region and is a source of many crucial micro as well as macro nutrients which are critical components of the human food. Although *A. hortensis* L. does not need much input to cultivate as vegetable still it is not grown in organized farming due to less economic value at present. Therefore it remains grossly underutilized for human consumption as vegetable. The present study has advocated that the rich mineral contents of *A. hortensis* L. make it very suitable vegetable candidate in the entire Ladakh region.

Table5. 1: Mineral elements (mg/g) estimated in the study population of *Atriplex hortensis* L.

Sr. no.	Locations	Ca	Mg	Zn	Fe	Na	Al	K
1	Chiktan	0.995±0.004^{abc}	0.176±0.002^{a}	0.251±0.003^{de}	0.191±0.003^{a}	0.187±0.002^{a}	4.643±0.560^{a}	1.557±0.050^{f}
2	Agham	0.983±0.015^{abc}	0.201±0.002^{b}	0.225±0.002^{c}	0.185±0.001^{a}	0.195±0.001^{b}	5.263±0.003^{b}	1.674±0.005^{h}
3	Soad	1.068±0.004^{abcd}	0.319±0.003^{h}	0.157±0.004^{a}	0.241±0.002^{a}	0.211±0.003^{d}	5.423±0.002^{b}	1.340±0.001^{b}
4	Khardong	1.110±0.020^{bcd}	0.273±0.003^{g}	0.265±0.003^{ef}	0.219±0.002^{a}	0.211±0.003^{d}	6.883±0.002^{de}	1.445±0.004^{d}
5	Hunder	1.317±0.001^{cde}	0.222±0.001^{d}	0.354±0.003^{h}	0.214±0.002^{a}	0.213±0.001^{d}	7.121±0.001^{ef}	1.616±0.001^{g}
6	Akchamal	0.687±0.508^{a}	0.235±0.001^{e}	0.293±0.001^{g}	0.213±0.003^{a}	0.228±0.001^{e}	6.796±0.003d^{e}	1.722±0.002^{i}
7	Shenam	0.744±0.001^{ab}	0.178±0.002^{a}	0.230±0.001^{c}	1.071±0.795^{b}	0.193±0.004^{b}	6.548±0.005^{d}	1.414±0.003^{c}
8	Gonpa	0.850±0.007^{ab}	0.247±0.002^{f}	0.199±0.001^{b}	0.208±0.002^{a}	0.211±0.002^{d}	12.947±0.006^{h}	1.643±0.003^{gh}
9	Murghi	0.966±0.001^{abc}	0.223±0.002^{d}	0.258±0.002^{def}	0.190±0.001^{a}	0.201±±0.001^{c}	7.660±0.020^{g}	1.493±0.003^{e}
10	Pakskum	1.697±0.002^{e}	0.375±0.001^{j}	0.354±0.002^{h}	0.261±0.001^{a}	0.304±0.001^{f}	5.871±0.001^{c}	1.827±0.002^{k}
11	Udmaru	1.036±0.002^{abcd}	0.203±0.001^{b}	0.274±0.001^{fg}	0.191±0.001^{a}	0.211±0.001^{d}	5.383±0.003^{b}	1.779±0.001^{j}
12	Diskit	1.128±0.001b^{cd}	0.216±0.002^{c}	0.202±0.001^{b}	0.203±0.002^{a}	0.212±0.002^{d}	7.391±0.007f^{g}	1.381±0.001^{bc}
13	Tangyar	1.563±0.006^{e}	0.334±0.001^{i}	0.239±0.023^{cd}	0.249±0.001^{a}	0.213±0.002^{d}	6.717±0.001d^{e}	1.717±0.001^{i}
14	Khungru	1.436±0.002^{de}	0.315±0.002^{h}	0.496±0.001^{i}	0.716±0.002^{ab}	0.184±0.002^{a}	7.475±0.003f^{g}	1.250±0.001^{a}
	Total	1.113±0.310	0.251±0.061	0.271±0.083	0.311±0.306	0.213±0.028	6.866±1.940	1.561±0.173

Sr. no.	Locations	P	S	Si	B	Cu	Se
1	Chiktan	0.174±0.006[a]	69.133±0.025[i]	0.132±0.003[b]	0.120±0.026[f]	0.046±0.003a	0.004±0.001a
2	Agham	0.395±0.507[a]	61.817±0.025[a]	0.057±0.0005[f]	0.128±0.002[f]	0.057±0.001[d]	0.036±0.002[d]
3	Soad	0.241±0.001[a]	62.207±0.025[d]	0.208±0.003[d]	0.017±0.002[abc]	0.049±0.002[ab]	0.078±0.002[h]
4	Khardong	0.157±0.005[a]	61.887±0.025[c]	0.166±0.001[c]	0.005±0.002[a]	0.051±0.002[bc]	0.046±0.002[e]
5	Hunder	0.244±0.003[a]	62.847±0.025[e]	0.222±0.002[d]	0.012±0.001[ab]	0.051±0.001[bc]	0.089±0.003[i]
6	Akchamal	0.323±0.004[a]	62.733±0.15[e]	0.143±0.006[bc]	0.014±0.001[abc]	0.048±0.001[ab]	0.086±0.000[i]
7	Shenam	0.241±0.002[a]	66.667±0.006[g]	0.095±0.003[a]	0.023±0.001[abcd]	0.045±0.004[a]	0.036±0.002[d]
8	Gonpa	0.324±0.003[a]	81.433±0.025[m]	0.137±0.001[h]	0.024±0.001[abcde]	0.055±0.001[cd]	0.019±0.001[b]
9	Murghi	0.147±0.001[a]	68.163±0.021[h]	0.147±0.002[bc]	0.029±0.001[bcde]	0.048±0.001[ab]	0.060±0.001[g]
10	Pakskum	0.304±0.004[a]	70.937±0.031[j]	0.289±0.001[e]	0.026±0.001[abcde]	0.049±0.001[ab]	0.025±0.001[c]
11	Udmaru	0.191±0.002[a]	53.090±0.020[b]	0.131±0.001[b]	0.035±0.001[cde]	0.050±0.001[ab]	0.037±0.001[d]
12	Diskit	0.162±0.001[a]	74.883±0.006[l]	0.128±0.002[b]	0.039±0.002[de]	0.048±0.001[ab]	0.051±0.002[f]
13	Tangyar	0.221±0.003[a]	63.677±0.006[f]	0.270±0.001[e]	0.034±0.001[cde]	0.047±0.002[ab]	0.024±0.001[c]
14	Khungru	0.316±0.002[a]	72.987±0.006[k]	0.275±0.001[e]	0.045±0.001[e]	0.047±0.001[ab]	0.044±0.001[e]
	Total	0.246±0.135	62.196±18.682	4.585±16.072	0.039±0.037	0.049±0.004	0.046±0.025

Table 5.2 Pearson correlation among mineral elements.

	Ca	Mg	Zn	Fe	Na	Al	K	P	S	Si	B	Cu	se
Ca	1	.697**	.463**	-.076	.442**	-.160	.119	.010	.123	-.114	-.073	-.090	-.158
Mg		1	.309*	-.055	.587**	.048	.046	.115	.252	-.231	-.378*	-.068	.005
Zn			1	.234	.125	-.053	-.052	.128	.159	-.156	-.084	-.179	.088
Fe				1	-.213	.002	-.381*	.059	.143	-.116	-.104	-.342*	-.081
Na					1	-.044	.565**	.102	.169	-.170	-.340*	.019	.001
Al						1	-.059	.096	.449**	-.232	-.382*	.355*	-.064
K							1	.154	-.259	.184	.095	.267	-.208
P								1	-.236	.312*	.119	.271	-.005
S									1	-.933**	-.571**	-.497**	-.033
Si										1	.669**	.584**	-.104
B											1	.144	-.506**
Cu												1	-.010
se													1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Present results indicate the significant variability in the mineral elements concentration in different germplasm collections. This variability could be due to genotypic difference in the species. Genotypic difference in legumes has been found to vary concentration and content of mineral in the previous studies.

Objective IV

Assessment of genetic diversity of *Atriplex hortensis* L. among indigenous populations of Ladakh using RAPD markers.

Materials and Methods

Study sites and sampling: Forty five sites were surveyed in order to cover diverse growing habitats which represent the richly capable yet relatively stable primitive environment in the sampled regions in which fiveteen populations of Atriplex from Ladakh regions was found Table (4.1). Seventy five individuals of young leaves were selected randomly for molecular analysis. Five Samples were taken in each population (Table 4.1).

Table 4.1 Geographical locations and sample sizes of *Atriplex hortensis*

S.No	Population name	Population code	Sample Size	Altitude (m)	Longitude (E)	Latitude (N)

1	Khardong	A	6	4109	077° 39.487	34° 33.822
2	Tangyar	B	8	3915	077°52.259	34° 15.168
3	Agham	C	9	3335	077° 49.877	34° 19.706
4	Murghi	D	5	3201	077° 32.643	34° 45.516
5	Hunder	E	6	3169	077° 27.688	34° 35.213
6	Udmaru	F	5	3129	077° 26.180	34° 37.627
7	Diskit	G	11	3117	077° 32.686	34° 33.210
8	Gonpa	H	5	3666	077° 35.560	34° 11.026
9	Shenam	I	9	3477	077° 34.766	34° 09.550
10	Chiktan	J	8	3252	076°30.432	34°28.166
11	Achakmal	K	6	2888	076°09.449	34°33.220
12	Pakskum	L	11	2881	076°09.443	34°33.120
13	Soad	M	7	2860	076° 10.305	34°33.974
14	Panamik	N	5	3184	077° 32.304	34° 46.963
15	Khungru	O	9	3623	077° 26.556	34° 17.003

RAPD analysis: Total genomic DNA was extracted from *Atriplex* leaves using a cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) with minor modifications. The quantity and quality of isolated total genomic DNA was determined using 0.8% agarose gel electrophoresis in 0.5 × TAE buffer for mobility related to known concentrations of lambda DNA. Thirteen primers of sets A, B, C, D and E, displaying reliable banding patterns, were used for PCR amplification (Dorda et al., 2005). Amplifications were performed in BIO RAD C 1000 Touch^{TM} Thermal cycler, each consisting of 94 °C for denaturation step (1 min), 36 °C annealing step (2 min), and a 72 °C extension step (1min). Amplification product were electrophoresed on 1.0% agarose gel (Life Science technologies, USA) and run at constant voltage (80V) in 1× TAE for 3 h, visualized by staining with ethidium bromide (0.5 µg ml-1) and a total of 2.5 µl loading buffer (6x) was added to each reaction before electrophoresis. After electrophoresis, the gels were documented on a gel documentation system (Alpha Innotech, Alphaimager, USA). Some RAPD-PCR reactions were repeated three times to ascertain the reproducibility of banding pattern.

Data analysis: The resulting presence/absence binary data matrix was analyzed using POPGENE version 1.31 (Yeh et al., 1999). Jaccard's similarity coefficient (J) was used to calculate genetic similarity between pair of individuals. The similarity matrix was subjected to cluster analysis by unweighted pair group method with arithmetic mean (UPGMA) (Rohlf, 1992). POPGENE software version 1.31 was used to describe the structure of studied populations with their geographic location. Observed number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL) and percentage polymorphic loci (PPL) across were analyzed

(Zhao et al., 2006). The non-parametric analysis of molecular variation (AMOVA) (Excoffier et al., 1992) was performed using squared Euclidean distances among all samples to partition the variation into two hierarchical levels; individual and population. GenAlEx software was used to calculate a principal coordinates analysis (PCA) that plots the relationship between distance matrix elements based on their first two principal coordinates (Peakall and Smouse, 2001). According to Prevost and Wilkinson (1999) the resolving power (Rp) of a primer is: Rp = Σ *IB* where *IB* (band informativeness) takes the value of: 1–[2* (0.5–P)], P being the proportion of the 96 genotypes containing the band. Nei's analysis of gene diversity among population (Nei, 1978) was carried out with counting total genetic diversity (Ht), within species diversity (Hs), genetic diversity between populations (Gst) and estimation of gene flow (Nm) from parameters. Fst index (Wright, 1951) was measured via this formula (Lynch and Milligan, 1994).

Results

The thirteen primer used in this study rendered 246 bands across seventy five *Atriplex* individuals, with 13 to 29 bands per primer, 18.92 as average (Table 4.2) and their size range from 260 to 2340 bp. Of the 246 bands, 229 (93%) were polymorphic and sixteen (7%) were monomorphic among the *Atriplex* individuals. RAPD genetic diversity analysis revealed the highest values of Nei's genetic diversity (0.25), Shannon information index (0.38) and polymorphic loci (87.37%) among accession from tangyar population and lowest values of Nei's genetic diversity (0.11), Shannon information index (0.17) and polymorphic loci (31.35 %) among accession from Akchamal population (Table 4.3). Nei's (1978) classified levels of genetic distance at < 0.05 as low, between 0.05 and 0.15 as medium and > 0.15 as high. Thus, the Akchakmal accession varied in narrow range while, the Tangyar accessions were more diverse.

Table 4.2 Sequences of the thirteen primers, with the number of scorable amplified and polymorphic bands

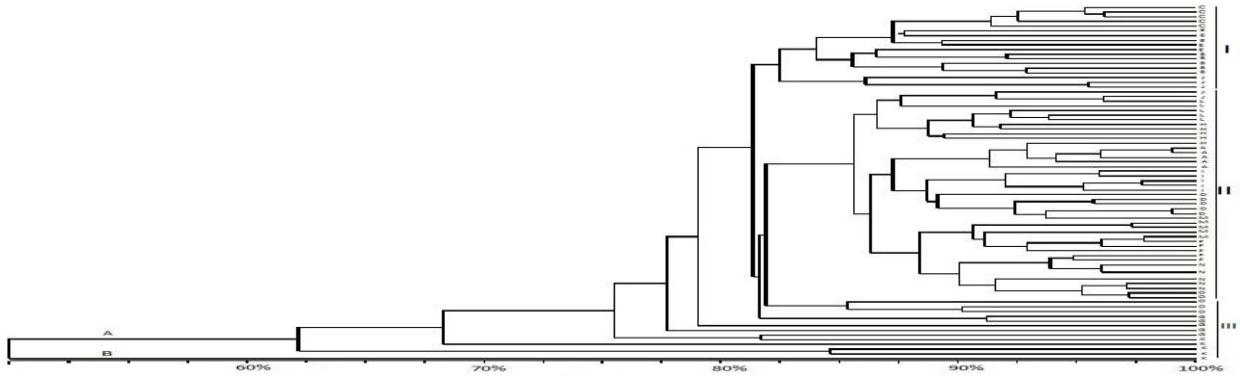
Primer	Sequence	No. Of amplified bands	No. of polymorphic bands
OPA-02	TGCCGAGCTG	18	17
OPA-05	AGGGGTCTTG	17	17
OPA-09	GGGTAACGCC	15	14
OPB-01	GTTTCGCTCC	20	19
OPB-03	CATCCCCCTG	19	18
OPB-06	TGCTCTGCCC	17	15
OPC-07	GTCCCGACGA	24	22
OPC-08	TGGACCGGTG	19	18
OPC-15	GACGGATCAG	16	16

OPD-08	GTGTGCCCCA	18	17
OPD-11	AGCGCCATTG	21	19
OPD-15	CATCCGTGCT	29	24
OPE-12	TTATCGCCCC	13	13
Range		13-29	13-22
Mean		18.92	17.62
Total		246	229

Table 4.3: Estimation of genetic variation statistics for all loci of RAPD among the Atriplex genotypes.

Markers	**Sampling sites**	**Sample size**	**H (mean + SD)**	**I (mean + SD)**	**PPL**
RAPD	Khardong	5	0.12 ± 0.17	0.18 ± 0.26	36.72
	Tangyar	5	0.25 ± 0.17	0.38± 0.22	87.37
	Agham	5	0.16 ± 0.18	0.24 ± 0.27	51.41
	Murghi	5	0.19 ± 0.17	0.29 ± 0.26	61.58
	Hunder	5	0.22 ± 0.19	0.36 ± 0.27	68.36
	Udmaru	5	0.20 ± 0.18	0.31 ± 0.26	62.15
	Diskit	5	0.20 ± 0.18	0.31 ± 0.27	62.71
	Gonpa	5	0.17 ± 0.18	0.26 ± 0.26	54.80
	Shenam	5	0.13 ± 0.18	0.20 ± 0.27	37.29
	Chiktan	5	0.13 ± 0.19	0.20± 0.27	36.72
	Achakmal	5	0.11 ± 0.16	0.17 ± 0.16	31.35
	Pakskum	5	0.22± 0.18	0.34 ± 0. 21	72.41
	Soad	5	0.17 ± 0.19	0.25 ± 0.28	52.42
	Panamik	5	0.20± 0.18	0.30 ± 0.27	62.59
	Khungru	5	0.12 ± 0.17	0.19 ± 0.26	36.28
Mean			**0.17**	**0.28**	**-**

was presented in (Figure 4.1). The dendrogram showed four main clusters (I, II, and III). The cluster I consisted of samples from populations Agyam, Hunder, Tangyar and Chiktan. Cluster II consisted of samples of population Pakskum, Gonpa, Khardong, Shenam, Murghi, Soad, Udmaru, Panamik, and khungru Cluster III consisted of Diskit, Akchamal, and some populations of khungru.



Genetic similarity

Figure 4.1 Dendogram illustrating genetic relatedness among 75 Atriplex individuals generated by UPGMA cluster Analysis based on 229 RAPD-bands.

The Genetic differentiation among populations (Gst) was, estimated to be 0.26, which indicated that 26% of the genetic variability was distributed among populations (Table 4.3). The level of gene flow (Nm) was estimated to be 1.53 individual per generation between populations. According to results of AMOVA analysis, there were highly significant (P <0.001) genetic differences within and among population of *A. hortensis*. Of the total genetic diversity, 67% was attributed to be among populations while the rest 33% was attributed to the differences within populations (Table 4.4).

Table.4.4 AMOVA analysis based on RAPD among the populations of *Atriplex*.

Source of Variation	Among population	Within population	Total
d.f.	14	60	74
Marker			
S.S.D.	46.917	34.000	80.917
Variance component	0.517	0.391	0.908
Percentage	67%	33%	100 %
P-value	< 0.001	< 0.001	< 0.001

Where d.f.= degree of freedom, S.S.D.= sum of square deviation, P-value = probability of null distribution.

In this study, we investigated variability among populations. Based on RAPD analysis, we revealed low genetic variation within the population and high genetic differentiation among the population. Therefore, the species concerned in this investigation have exhibited, in previous study, a large variability in agro-morphological traits. Thus the present work confirm that this difference among and within population have genetic basis and provides base to select the potential traits in breeding programs by genetic markers coupled with agro-morphological traits.